Original Article

Effect of Prevalence of E-Learning and Excessive Use of Disinfectants to Bacterial Contamination of Mobile Phones and Computer Keyboards during the COVID-19 Pandemic

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Abstract

Background: In Iraq, first confirmed cases of COVID-19 were reported in February 24, 2020. Because of applying of healthy quarantine, electronic learning was applied in order to prevent the potential spreading of COVID-19 in the educational institutions, using of mobile phone and computer increased and given a great opportunity for increasing the microbial contamination. **Aims:** Study effect of excessive use of e-learning and disinfectants on the bacterial contamination of mobile phones and keyboards during the Corona epidemic. **Methods and Materials:** 200 samples were collected from computer keyboards and mobile phones used for E-learning by undergraduate students in college of Pharmacy/University of Babylon, Iraq for the period from 1st April–15th June 2021, during COVID-19 pandemic. Isolates were diagnosed by VITEK 2 system. Agar-well diffusion method was used to evaluate the antibacterial activity of some disinfectants included sodium hypochlorite (NaOCl) (5% (, ethanol 70%, ethanol 99%, chlorhexidine gluconate 4% w/v, and chloroxylenol 4.8 w/v. **Results:** 308 isolates were obtained included 183 isolates from mobile phones and 125 isolates from computer keyboards. Antibacterial activity was varied depending on bacterial isolates, type and concentration of disinfectant. Sodium hypochlorite 5% revealed highest antibacterial activity against all studied bacteria followed by ethanol 70% and chlorhexidine gluconate 4% w/v, whereas chloroxylenol 4.8 w/v and ethanol 99% have the lowest activity. **Conclusions:** In light of the world's preoccupation with the Corona pandemic, the use of mobile phones and computers for the purpose of e-learning increased, which caused an increase in bacterial contamination of these devices, which required drawing attention to this aspect.

Keywords: Bacterial contamination, computer keyboards, COVID-19, E- learning, mobile phones

Introduction

In Iraq, the first confirmed case of COVID-19 was reported in February 24, 2020, day after day, more cases were reported. Thus, a critical decision for applying of preventive measures became necessary. The protection of students, teachers, and educational facilities is an essential goal in order to prevent the potential spreading of COVID-19 in school and university environments.

Using of computer in the university's environment is still increasing due to its essential roles through the regularly using of internet, mailing and social media. Recently, electronic learning (E-learning) has been applied as a result of COVID-19 pandemic, which caused over use of computer and mobile phone during home's quarantine. Thus, most universities globally applied e-learning as a perfect replacement which made the computer and mobile phone the main instruments for this purpose. The overuse of these instruments without doing routine disinfection gives a great opportunity for increasing their bacterial and viral contamination.

It is interesting that, no clear legislation or recognized guidelines have been formulated on the biological hazard

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associated with the computer and mobile phone, yet. The wide spreading of multiple-user computer keyboards in different public locations for different reasons may represent a hidden transmission rout for bacterial diseases which make the computer keyboard acts as a reservoir for the pathogens and contribute to cross-transmission.[1]

Mobile phones are long range, portable electronic devices that had essential roles in our life, work, and communications which provides a chance for exposure it to wide spectrum of microbes to become a dangerous reservoir and carrier for microbes especially that correlated with skin infections. The temperature and moisture of human body and heat generated by mobile phone introduce an optimum conditions for microbial harboring and transmission.[2]

Recently, at the time of COVID-19 pandemic, the computer and mobile phone became the most important means for e-learning, virtual meetings, conferences, and workshops worldwide. In Iraq, both ministry of education and ministry of higher education and scientific research applied e-learning in the universities and institutes all over Iraq, which required over use of computer and mobile phone to communicate with the virtual lectures, courses, and examinations; this was accompanied with an increase in reported cases of COVID-19.

COVID-19 is a viral disease caused by SARS-COV-2 virus. The studies confirmed that SARS-COV-2 has a high ability to survive on inanimate surfaces for relatively long periods and confirmed that the regularly disinfection of the surfaces by using appropriate disinfectant is a main protective measure to inactivate this virus during seconds.[3,4]

Strength of antimicrobial activity for disinfectant is necessary to enhance its controlling of the microbes and prevention of disease spreading and to achieve a total disinfection of the surfaces.[5]

During this critical period with increasing risk of any viral pandemic, fully understanding is needed for the hazard of any source of contamination, especially which is linked with our daily life. Up to our best knowledge, there is no work has been studied the bacterial contamination on mobile phone and computer keyboard used by the undergraduate students and lecturers during the E-learning in Iraqi universities, yet. This study aimed to introduce a clear profile of bacterial contamination associated with mobile phone and computer keyboard and evaluate the efficacy of common disinfectants during electronic learning.

Materials and Methods

Samples collection and laboratory diagnosis

A total of 200 samples were collected: 100 swabs from computer keyboards and 100 swabs from mobile phone used for e-learning by the undergraduate students in the College of Pharmacy, University of Babylon, Iraq for the period from 1st April–15th June 2021, during the quarantine period in the time of COVID-19 pandemic. A transport swab has been moisturized by sterile normal saline and wiped firmly over the entire surface of computer keyboards and mobile phones. These swabs have been transported immediately to the laboratory by transport media and inoculated on MacConkey agar and blood agar and incubated aerobically at 37ºC for 24–48 h. After some essential tests, the pure bacterial isolates were diagnosed by VITEK 2 system.

Preparation of bacterial suspension

An inoculum was prepared by adding 2–3 pure colonies of already diagnosed bacterial isolate to 5 mL of sterile Brain Heart Infusion broth and incubated at 37ºC for 18 h after incubation period, 1 mL of this broth culture was diluted by sterile normal saline in sterile plane tube to produce a standard bacterial suspension with moderate turbidity equal to McFarland standard tube.

Efficacy of disinfection against bacteria isolated from computer keyboard and mobile phone

Preparation of chlorhexidine gluconate dilution

The dilution was prepared according to the instructions of manufacturing company by adding 1 mL of chlorhexidine gluconate (CHG) (4% w/v) made up to 100 mL with sterile distilled water to produce dilution (1%).

Preparation of chloroxylenol dilution

According to the instructions of the manufacturing company, preparation of chloroxylenol (PCMX) (4.8 w/v) was concentrated solution used for preparing dilution 1:20 by diluting 1 mL of PCMX in 20 mL of sterile distilled water to produce 5% dilution.

Preparation of sodium hypochlorite dilutions

NaOCl 5% was concentrated solution (stock). Four dilutions were prepared from it included:

Dilution 1:2 (addition 100 mL of sodium hypochlorite [NaOCl] 5% to 200 mL sterile distilled water)

Dilution 1:4 (addition 100 mL of NaOCl 5% to 400 mL sterile distilled water).

Dilution1:6 (addition 100 mL of NaOCl 5% to 600 mL sterile distilled water)

Dilution 1:8 (addition 100 mL of NaOCl 5% to 800 mL sterile distilled water)

Antibacterial activity of disinfectants against bacteria isolated from computer keyboard and mobile phone

Agar-well diffusion method used to evaluate the antibacterial activity of four common disinfectants included NaOCl, ethanol 70%, ethanol 99%, CHG and

PCMX, Table 1 against 12 bacterial species isolated from computer keyboard and mobile phone: *Staphylococcus aureus*, Coagulase Negative Staphylococci (CoNS), *Enterococcus faecalis*, *Strptococcus pyogenes; Viridans Streptococci; Bacillus cereus*, *Escherichia coli*, *Psedomonas aeruginosa*, *Klebsiella pneumonia*e, *Acinetobacter baumannii*, *Proteus mirabilis*, and *Salmonella* sp.

A sterile swab was used to obtain an inoculum from the bacterial suspension and streaked on a Mueller-Hinton agar. A hole with a diameter of 6 mm has been punched by a sterile cork borer (No. 6) on the streaked plate. Approximately 200 µL of each disinfectant was introduced into each hole (carried out in duplicates and the mean of the duplicate results were taken). A negative control was prepared by putting 200 µL of sterile distilled water in one of holes. Pre-diffusion time was allowed for one hour, after which the plates have been incubated at 37°C for 18 h. Diameter of inhibition zone was measured in millimeter.

Ethical approval

The study did not include collecting samples from humans or animals. For collecting swabs from mobile phones and computers, permission and verbal consent were obtained from the owners (students of the College of Pharmacy, University of Babylon). The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee, according to the document number (56, January 18, 2021) to get this approval.

Results

Out of 100 samples collected from computer keyboards, only 73 (73%) samples yielded bacterial growth, 5 (5%) samples have fungal growth and 22% have no growth, whereas all 100 (100%) samples collected from mobile phones yielded bacterial growth [Figure 1].

In the present study, laboratory diagnosis of bacterial isolates was achieved by VITEK 2 system. Table 2 shows the bacterial species isolated from computer keyboard and their percentages.

Table 3 shows the bacterial species isolated from mobile phone and their percentages.

Table 4 shows that all assayed disinfectants revealed a clear inhibitory action against studied bacteria isolated from computer keyboard and mobile phone.

Figure 2 shows the diameters of inhibition zones for the studies disinfectants against Gram-negative and Gram-positive bacteria.

Figure 3 shows the antibacterial activities of four dilutions of NaOCI in the present study.

Discussion

The keyboard could possibly provide the heat and moisture for long enough durations for bacteria to persist on these surfaces due to the difficulty of its cleaning and disinfection. Unfortunately, people have paid attention for the risk of radiation by mobile phones and ignore microbial contamination resulting from direct contact of this device with skin, hands, mouth, nose, face, ears and sometimes hair. In addition to that, the dirt and sweat accumulation in the body slits of mobile phone together with its cover and its accessories get various microbes that may be more than what are found in toilet seats and rightly

Table 1: Disinfectants and their concentrations used in the present study

The Disinfectant	Abbreviation	Concentration	Description
Sodium hypochlorite 5% (50 mg/mL)	NaOCl	5% Stock	Disinfectant
Ethanol 70%	E70%	70% Stock	Disinfectant, Antiseptic
Ethanol 99%	E99%	99% Stock	Disinfectant, Antiseptic
Chlorhexidine gluconate 4% w/v (Hibitane)	CHG	1:100 Dilution	Antiseptic; Disinfectant
Chloroxylenol (Dettol) 4.8 w/v	PCMX	1:20 Dilution	Antiseptic; Disinfection

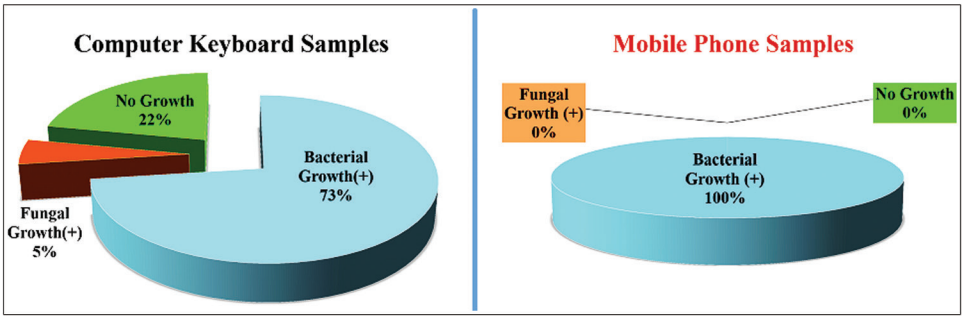

Figure 1: The percentages of bacterial growth on the computer keyboards and mobile phones in the present study

Table 2: Contaminated bacteria isolated from the computer keyboards

The bacteria		No.	%
Gram-Positive (48 isolates)	*Staphylococcus aureus*	20	16
	Enterococcus faecalis	15	12
	Coagulase Negative Staphylococci (CoNS)	11	8.8
	Bacillus cereus	2	1.6
Gram Negative (77 isolates)	*Escherichia coli*	32	25.6
	Psedomonas aeruginosa	19	15.2
	Klebsiella pneumonia	10	8
	Acinetobacter baumannii	9	7.2
	Proteus mirabilis	6	4.8
	Salmonella sp.	1	0.8
Total		125	

Table 3: Contaminated bacteria isolated from the mobile phone

The Bacteria		No.	%
Gram-Positive (156 isolates)	Coagulase Negative Staphylococci (CoNS)	55	30
	Staphylococcus aureus	46	25.1
	Enterococcus faecalis	32	17.5
	Streptococcus pyogenes	9	4.9
	Viridance Streptococci	9	4.9
	Bacillus cereus	5	2.7
Gram-Negative(27 isolates)	*Escherichia coli*	27	14.8
Total		183	

Table 4: Antibacterial activity of disinfectants against bacteria isolated from computer keyboard and mobile phone in the present study

No.	Bacteria	(diameter of inhibition zone by mm)				
		NaOCl 50%	E70%	E99%	CHG	PCMX
1.	*Staphylococcus aureus*	48	32	15	46	17
2.	CoNS	49	31	15	41	17
3.	*Enterococcus faecalis*	45	29	0	35	29
4.	*Streptococcus pyogenes*	47	29	12	37	24
5.	*Viridans Streptococci*	47	30	10	45	26
6.	*Bacillus cereus*	46	0	0	0	0
7.	*Escherichia coli*	46	27	12	24	20
8.	*Psedomonas aeruginosa*	45	0	0	0	0
9.	*lebsiella pneumonia*	47	29	13	29	20
10.	*Acinetobacter baumannii*	43	24	0	28	22
11.	*Proteus mirabilis*	44	20	14	24	20
12.	*Salmonella sp.*	46	22	16	29	19

is called as "technological Petri-dish" for thousands of bacteria.[6-8]

Isolation and identification

Among 173 samples revealed bacterial growth in this study, 308 bacterial isolates were obtained which indicated a mixture bacterial isolation. Through our personal observations, computer keyboard and mobile phone are vulnerable for different contaminants such as respiratory secretions (by coughing and sneezing), mucus, saliva, blood, feces, urine, and pus which may be excreted by persons with infectious diseases.

Bacterial isolation from computer keyboard

During COVID-19 pandemic, using of computers by university students has increased at home, sometimes laptop was being moved from one place to another, or used in inappropriate places such as the kitchen, garden, places with poor cleanliness, or dirty. So, computer keyboard became more vulnerable for the contamination with food,

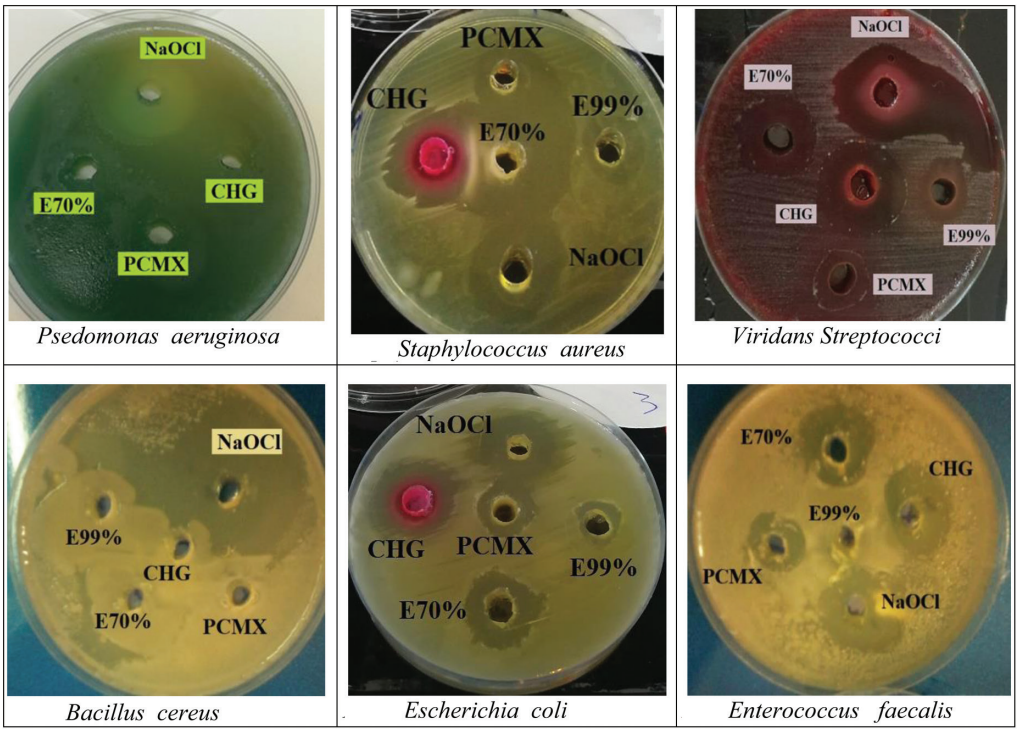

Figure 2: In the present study, diameters of inhibition zones varied according to the type and concentration of disinfectant, bacterial isolates, and its intrinsic resistance. NaOCl = Sodium Hypochlorite 5%, E70% = Ethanol 70%; E99% = Ethanol 99%; HG = Chlorhexidine Gluconate 4% w/v; PCMX = Chloroxylenol

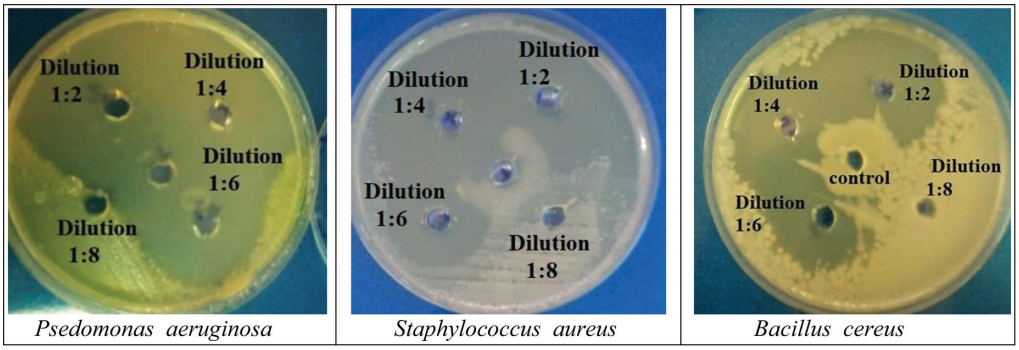

Figure 3: In the present study, four dilutions of NaOCl 5% were examined to evaluate their antibacterial activity; dilutions (1:2, 1:4, 1:6) have high antibacterial activity against studied bacteria, whereas dilution (1:8) has no antibacterial activity

oils, sweat, saliva, mucus, and dirt making it a dangerous reservoir for different microbes as well as SARS-C0V-2.

As detailed in Table 2, Gram-negative bacteria are most predominantly present in the computer keyboard and majority belonged to Enterobacteriaceae represented an indicator for the fecal contamination. Gram-negative bacteria have the ability to persist on inanimate surfaces greater than gram-positive bacteria. *E. coli*, *Klebsiella* spp., *P. aeruginosa,* and *Acinetobacter* spp. can survive for months, whereas *Salmonella* spp. and *Proteus* spp. can survive for 1–2 days.[9,10]

Among Gram-negative bacteria in the current study, *E. coli* is the dominant bacterium followed by *P. aeruginosa* and *K. pneumoniae*. *E. coli* is an intestinal normal flora and fecal coliform bacteria and refers for fecal contamination and a low level of hand and computer hygienic practices. *P. aeruginosa* is distributed widely of environments because of its easy adaptation to the environmental changes, its ability to tolerate a range of physical conditions, and its ability to thrive in diluted sterilizers and disinfectants. Both *E. coli* and *P. aeruginosa* have ability to produce biofilm. *K. pneumoniae* may be spreading through respiratory secretions by coughing and sneezing.[11,12] Among gram-positive bacteria, *S. aureus* has the highest percentage(16%) [Table 2].

Bacterial isolation from mobile phone

During the COVID-19 pandemic, the excessive use of mobile phones became common in inappropriate places,

bathroom and toilet, which made it a means of microbial transmission among places and people. As well as, using decorated cover and accessories with rough decorations (slits or hinges) which introduce a greater chance of microbial accumulation.

As detailed in Table 3, in mobile phone, Gram-positive bacteria are predominant. Coagulase Negative Staphylococci have highest percentage may be because of its ability for biofilm formation which help it for adherence, colonization, and persistence on the inanimate surfaces for months and protect it from disinfectants and antibiotics.[13,14]

As shown in Tables 2 and 3, *B. cereus* is an uncommon but aggressive pathogen, isolated from both computer keyboard and mobile phone. The assessment of the original source of contamination with *Bacillus* spp. is often difficult because its spores are ubiquitous in the air, water, soil, feces, indoor air for long period and other environments.[15]

Antibacterial activity of disinfectants against bacteria isolated from computer keyboard and mobile phone

The disinfection is an elimination of most pathogenic microbes by destroying or inhibiting the growth of bacteria, viruses, and most fungi, except bacterial spores. Disinfection is applied on inanimate surfaces, usually by chemical solutions but it is not necessarily mean killing of all bacteria, but may reduce bacterial load to a level that does not harm health. Disinfection may be effected by various factors and can nullify or limit its efficacy, such as prior cleaning of surface; organic and inorganic load; type and level of microbial contamination; concentration of disinfectant; exposure time to the disinfectant; physical nature of the surface (crevices or hinges); biofilm formation by bacteria; and environment conditions of the disinfection process (temperature, pH and humidity). Prior cleaning is an essential step before disinfection by removing of visible soil, organic and inorganic material, from surfaces usually by water and detergents. Organic or inorganic materials may be acting as a physical barrier protect bacteria and interfere with the efficacy of disinfection. Antisepsis is an elimination of most pathogenic bacteria, viruses, and most fungi except bacterial spores, on living surfaces. Antiseptic is a germicidal used with living tissues and skin and not for surface whereas disinfectant is a germicidal used only to inanimate surfaces and not used for skin antisepsis. Duration of exposure must be considered because the surface must be exposed to the disinfectant for the suitable minimum contact time.[5,16,17]

As detailed in Table 4, antibacterial activity varied according to the type and concentration of disinfectant, bacterial isolates and its virulence factors and intrinsic resistance factors.

In the present study, NaOCl has the highest antibacterial activity followed by CHG and ethanol 70% whereas PCMX and ethanol 99% have the lowest activity.

Antibacterial activity of sodium hypochlorite

NaOCl has the highest diameters of inhibition zones against all studied bacterial. This finding is in agreement with many previous studies.[18,19]

NaOCl is one of chlorine-releasing compounds which has an effect against bacteria, fungi, enveloped and non-enveloped viruses. Prior cleaning is required for heavily soiled surfaces before using it because NaOCl often inactivated by organic soils. The most common concentration of NaOCl solution as a household disinfectant is 1%–5% available chlorine.[20,21] The dilution of NaOCl by water produces its active ingredient "hypochlorous acid (HOCl)" which represents a fast-acting and strong antimicrobial agent, and is predominant in pH (4–7). The antimicrobial activity of NaOCl acts by different mechanisms including reacting with amino acids, fatty acids, sulfur-containing amino acids; degrading lipids, nucleic acids, enzymes and cell membrane components; neutralization of amino acids; oxidation and inhibition of essential bacterial enzymes and disruption cell metabolism, finally, causing severe cellular damages leading to the cell death.[19,22]

A study by Nizer *et al*.,[23] introduced a schematic summery for the targets by hypochlorous acid in Gram-negative bacterial cells which included cell envelopes, cell membrane proteins and transporters, disruption ATP production, attack on cell membrane lipids causing loss of membrane stability, disruption protein synthesis (sulfur-containing proteins), interference with DNA synthesis.

As shown in Table 4, both *P. aeruginosa* and *B. cereus* revealed full resistance without any inhibition zone against all assayed disinfectants except NaOCl. This finding is in agreement with important references[5,23,24] have demonstrated that Ethanol, CHG and PCMX have no effectiveness against *P. aeruginosa* and spore forming bacteria. The most acceptable explanation of this resistance is the possession of intrinsic resistance tools such as biofilm and spore formation. It is known that, *P. aeruginosa* has high ability to resist and tolerate most disinfectants and detergents by different mechanisms. Biofilm limits the disinfection efficacy because bacteria it provides resistance 1000 times for disinfectants and tolerance 100–1000 times for antibiotics more than those. outside.[24]

This finding is in agreement with many studies where NaOCl has been demonstrated as active bactericidal against biofilm forming bacteria.[25] In the present study, the resistance of *B. cereus* against most disinfectants

may be attributed to its ability for spore forming which introduce highest intrinsic resistance to chemical bactericidal because the spore coat and cortex act as a barrier to protect cellular contents and genome from inactivation agents.[26]

Conducting the current study, the use of NaOCl as a disinfectant increased by 58% in March 2020 compared to March 2019 due to the COVID-19 pandemic.[27] Recent studies have shown that SARS-CoV-2 has the ability to persist on inanimate surfaces for varying periods of hours to days but a 0.1% NaOCl solution is sufficient to inactivate SARS-CoV-2 by interacting with the viral outer lipid envelope. In addition to its virucidal activity against human immunodeficiency virus (HIV), Ebola virus, and Norwalk virus. Excessive use of NaOCl may lead to a gradual increase in bacteria resistance against it and other chlorine-based disinfectants.[20,27-29]

Through our observations, during COVID-19 pandemic in Iraq, disinfectant fogging with NaOCl became common for disinfection of large areas, high buildings, hard to reach surfaces, whole houses, schools, and universities. The fogging is performed by creating a large scale foggy spray to cover as large as possible area. NaOCl became the most common chemical solution used for disinfectant fogging in order to limit the viral infection. The people are preparing dilutions from the household bleach and water for fogging of indoor and outdoor included the houses, gardens, and cars. Sometimes, NaOCl dilutions are used for spraying individuals in crowded places. These are bad procedures, that need to more awareness about the appropriate concentration of NaOCl dilution with highest activity and lowest toxicity, as well as, it is must be known that, the direct exposure with chlorine-releasing solutions in inappropriate concentrations may be caused irritation in eyes, skin, and respiratory tract. On the other hand, using NaOCl solution for fogging outdoor is not recommended because of the potential reaction of NaOCl with organic substances in outdoor environment and it becomes ineffective.

The present study tried to test the most common aqueous dilutions of NaOCl used for disinfectant fogging in our country. Four dilutions were prepared from NaOCl 5% (stock solution) and sterilized distilled water included (1:2, 1:4, 1:6, 1:8 v/v) and have been examined in order to evaluate their antibacterial activity. As shown in Figure 3, three dilutions (1:2, 1:4, 1:6) have high antibacterial activity against studied bacteria, whereas the last dilution (1:8) has no antibacterial activity against most studied bacteria. This finding demonstrated that dilution of NaOCl bigger than six times is inappropriate for disinfection. According to this finding, dilution 1:6 is the recommended dilution of NaOCl 5% for disinfection because of its low toxicity and high antibacterial activity. This finding is in agreement with previous studies that recommended NaOCl dilution as a necessary attempt to reduce its toxicity while keeping its antibacterial activity.[30,31]

Antibacterial activity of chlorhexidine gluconate (CHG)

Chlorhexidine gluconate solution (Hibitane) is a widely applicable as antiseptic and disinfectant for general purpose. It can be used on skin, hands, wounds, burns, medical instruments, hard surfaces and used as mouthwash in order to prevent dental plaque, treatment of gingivitis and oral Moniliasis (Candidiasis). It has many products such as solution, soap, gel and mouthwash. Presence of blood or body fluids may reduce its activity.[16,17] In the present study, dilution 1:100 has been prepared from CHG (4% w/v) and assayed to evaluate its antibacterial activity. As detailed in Table 4, CHG revealed a clear antibacterial activity against all studied bacteria except *B. cereus* and *P. aeruginosa*. Gram-positive bacteria showed sensitivity more than gram-negative, with biggest diameter for *S. aureus* and smaller diameter for *E. coli* [Table 4]. This finding is in agreement with important references[5,16,17] have demonstrated that CHG has high antibacterial activity against Gram-positive bacteria and enveloped viruses and moderate activity against Gram-negative bacteria, fungi and non-enveloped viruses whereas it is less effective against *Pseudomonas* spp. and *Proteus* spp. and does not inactivate spore forming bacteria. Notably, a study by Fehr and Perlman[31] mentioned that CHG may have antiviral activity against coronavirus as a member of enveloped viruses group. Before using CHG, dilution to appropriate concentration with water or alcohol is necessary, distilled water or tap water can be used for this purpose. Because of its poorly absorption, CHG can bind strongly to a variety of substrates and different tissues with keeping its antibacterial activity, then it is releasing slowly with keeping of effective. Mechanism of action of CHG is by releasing positively charged chlorhexidine cation which binds to negatively charged bacterial cell walls. In high concentration, CHG will result a bactericidal effect by cell membrane disruption and cell death, whereas at low concentrations, it will show a bacteriostatic effect.[32]

Antibacterial activity of ethanol 70% and ethanol 99%

Ethanol is a type of alcohol used as disinfectant and antiseptic because of its broad spectrum germicidal activities as fungicidal, virucidal, bactericidal, and bacteriostatic activity against vegetative cells but not against the spores. It's activity as bactericidal faster than bacteriostatic and the effectiveness depends on how concentrated it is. Antibacterial action by ethanol includes protein denaturation and dissolving the lipid bilayer in bacterial cell membrane causing disruption of the membrane structure and function which permits water to leave the bacterial cell resulting in cell dehydration and cell death. On the other hand, among the alcohol-based products, ethanol has the least skin-irritant property.[33,34]

As detailed in Table 4, ethanol 70% has inhibition zones bigger than those for ethanol 99%. Both *B. cereus* and *P. aeruginosa* have full resistance against ethanol 70% and ethanol 99%, and this may explain that, in many cases, serious wound infections with pathogenic spore forming bacteria have occurred after surgery when ethanol has been used for sterilization of surgical instruments and operation theatre. Table 4 shows gram-positive bacteria that are more sensitive than gram-negative for ethanol 70%. This result may be attributed to the fact that, ethanol mechanism of action is targeting the proteins in bacterial cell membrane and cell wall. Gram-negative possess an outer membrane surrounded cell wall and acts as a barrier to the uptake of disinfectants as contrasted with gram-positive bacteria who have cell wall without an outer membrane.[5,35]

Ethanol(99%) has bactericidal effect less than aqueous dilutions of ethanol(70%) because ethanol 99% is a dehydrating agent while ethanol 70% is a denaturizing agent for bacterial proteins. Protein denaturation process become more quickly in the presence of water, but dilution below 50% may be decreased bactericidal activity. Thus, ethanol (60%–90%) v/v are the optimum concentrations for bactericidal activity. Ethanol(60%–80%) is a potent virucidal agent inactivating herpes, influenza virus, adenovirus, enterovirus, rhinovirus, and rotaviruses.[5,36]

Ethanol denatures protein by breaking the hydrogen bonds that link the hydrogen and oxygen atoms in different sites of the chain-like molecules, And then, forming new hydrogen bonds at these sites, causing protein molecule loses its specific shape and function. The best denaturation is by ethanol 70% as it is easily absorbed by cells. In higher concentrations of ethanol, the water is lower making absorption and penetration by bacterial cell difficult. So, protein denaturation and coagulation will be happened on the cell surface which prevents more penetration resulting temporary inactivation of bacteria. Usually, bacteria can survive these conditions.[25,33]

Through our observations, during COVID-19 pandemic, ethanol is the most likely candidate and most common using as a disinfectant. Many studies conducted with WHO recommended that ethanol was able to destroy coronaviruses. The mechanism of action of ethanol has a common essential step for inactivation of both bacteria and virus by dissolving the lipid layers in their membranes.[37]

Centers for Disease Control and Prevention(CDC) recommended hand washing with ethanol-based soap and water for 20-30s as a preferred disinfection procedure, although water is not readily available all the times. Ethanol spray or ethanol-based sanitizer may be less efficient in this time. Sometimes, people may not spray enough amount of ethanol to clean and disinfect the hands, because ethanol spray may evaporate before it was rubbed all over the hands completely especially outdoor, as well as, using ethanol spray may be not useful unless hand washing before if the hands are dirty or contaminated with chemicals.[38,39]

Antibacterial activity of chloroxylenol

Chloroxylenol is para-chloro-meta-xylenol (PCMX) and a chlorinated phenolic compound, Dettol is the most common brand name. It is commonly used as disinfectant and antiseptic agent for wounds, damaged skin, instruments, and surfaces. Because of its antimicrobial activity, it is used widely in cosmetics and soap. Its mechanism of action is by the inactivation of enzymatic systems and disruption of bacterial cell wall synthesis.[16,17,40]

As detailed in [Table 4], PCMX revealed antibacterial activity less than other disinfectants assayed in the present study. Staphylococci have the smaller diameter of inhibition zones than other gram-positive bacteria, while gram-negative bacteria have diameters less than gram-positive ranged. Notably, both *B. cereus* and *P. aeruginosa* have no inhibition zone.

This finding is in agreement with studies confirmed that PCMX is less effective than some other available agents. PCMX has bactericidal activity against Gram-positive bacteria but less effective against Staphylococci and Gram-negative bacteria, whereas it has no effective against *Pseudomonas* spp. and spore forming bacteria. On the other hand, PCMX has clear activity against enveloped viruses.[16,17] Aqueous dilutions of PCMX are vulnerable for microbial contamination during the dilutions preparing because of its sensitivity for contamination. Thus, its dilutions should be freshly prepared and the dilution process and storage required appropriate measures to avoid microbial contamination.[17,35]

Either with pandemic or without it, there several recommendations we must take into consideration in order to stay safe: Keeping hands clean is a critical procedure for limiting the microbial transmission to the electronic devices' users. During e-learning, routine disinfection and choosing an appropriate place to study may be the optimal daily action required. in order to improve the disinfection efficiency. Prior cleaning is an essential step before it, ethanol 70% can be used for disinfection of hands, mobile phone, and computer keyboard. In general, diluted NaOCl can be used for disinfection of hard surfaces in a suitable concentration, but the dilution 1:6 is the recommended, with keeping the safety precautions. It must be taken in consideration that using of NaOCl solution is not recommended for outdoor fogging due to the possible reaction of NaOCl with organic matter which makes it ineffective. On the other hand, spraying individuals by NaOCl dilutions for disinfection in crowded places is not be recommended, because it may be cause irritation in eyes, skin, and respiratory tract. Finally, more awareness is required of people, especially for university students, about the biological risks associated with excessive use of computers and mobile phones and importance of the regular sterilization and disinfection.

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Conflicts of interest
There are no conflicts of interest.

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